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FERMENTATION OF ORANGE JUICE AS AFFECTED BY THE ADDITION OF NITROGENOUS NUTRIENTS*

by
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INTRODUCTION

Nitrogen is an essential element for the nutrition, propagation, and fermentation processes of yeast (1,4). Compounds from which yeast will readily split off nitrogen in the form of ammonia are regarded as being the most easily assimilable (5,6,7). Amino acids, simple amides and polypeptides are increasingly difficult for yeast to assimilate (8,10,14).

Orange juice contains comparatively little of such nitrogenous substances as are necessary for the most favorable nutrition of cultivated yeast. When the amount of sugar necessary to produce a wine of 13 or more percent of alcohol by volume is added to the juice an actual deficiency of nitrogenous yeast food results.

Since wine yeasts are capable of utilizing either inorganic or organic sources of ammonia nitrogen, it was believed that addition of small amounts of ammonium carbonate or ground converted distillers' malt would stimulate the growth and activity of the yeast and shorten the period of fermentation. Data presented in this paper will show this to be the case. The quality of the fermented product was also found to be superior to that obtained when these nitrogenous nutrients were not used.

EXPERIMENTAL

Addition of Ammonium Carbonate to Orange Must: Lindner[11] and Pringsheim[2] have found the optimum nitrogen concentration of ammonium salts for maximum yeast growth to be 0.0321 percent, and the optimum for fermentation activity to be 0.008 percent. Preliminary experiments showed that the optimum range of ammonium carbonate for the stimulation of yeast activity was 0.9 to 1.0 grams per liter, representing 0.026 - 0.029 percent nitrogen, where sucrose had been added to the orange juice to increase the Brix to 24-27°.

In this work Florida Valencia orange juice to which sucrose had been added to increase the Brix to 24.4° was used. The sweetened juice had a pH of 3.97 and a total acidity of 0.75 grams per 100 cc, calculated as anhydrous citric acid. Ten samples of two liters each were used. To five of these ammonium carbonate was added at the rate of 0.9 grams per liter, which reduced the pH to 4.14 and the acidity to 0.73 grams per 100 cc. The remaining five served as controls.

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Ten yeast starters, each of 125 cc, were prepared. To each of five 150 cc flasks was added 125 cc of the sweetened juice. To another set of five similar flasks was added 125 cc of juice treated with both sugar and ammonium carbonate in the same proportions as in the two-liter batches. These two sets of flasks were inoculated with five different strains of yeast which had been grown for five days in an 18° Brix orange must, and were incubated for 48 hours at 20°C. (68°F.), the set without added carbonate being used to inoculate the control batches.

These starters were added to the two-liter batches which had been held at 10°C. (50°F.), and fermentation was carried out at room temperature, 16°-29°C. (60°-84°F.).

The data show that fermentation was appreciably accelerated by the use of ammonium carbonate. If the total losses in weight, caused by the evolution of carbon dioxide and corresponding directly with the increase in percent alcohol are plotted against time, as in Figure 1, it will be seen that for the first several hours there is practically no evolution of carbon dioxide (curves E and F). The curve for carbon dioxide losses then rises sharply. After several days the curve levels off and becomes nearly horizontal. The fermentable sugars in the must are nearly used up and the yeast cells now begin to utilize their reserve carbohydrates (glycogen)[12]. The phase of the fermentation which is of importance in the present investigation is the period of maximum yeast growth and activity, i.e., the period during which fermentation of the juice takes place.

If, now, we compare the curves during the period of maximum fermentation activity of the must to which ammonium carbonate was added (curves A, C, E) with those of the respective controls (curves B, D, F), it will be seen that the rate of fermentation was greater in the must containing the added nutrient salt. Inasmuch as the rate of alcohol production was greater in the treated must, it is probable that spoilage organisms could be more easily overcome, and in a shorter period of time. Wortmann[3], and Euler and Lindner[9] have shown that to produce quality wines the yeast should develop rapidly and produce alcohol quickly in order to overcome spoilage organisms in the shortest possible time. Organoleptic tests showed that musts to which ammonium carbonate had been added produced a wine having an aroma and taste superior to that produced by the controls.

To confirm the effects of the addition of ammonium carbonate to orange juice, the experiment was repeated with the five strains of yeast previously used and with three additional strains. In this second experiment the amounts of added sucrose and ammonium carbonate were increased. Sucrose was added to raise the Brix to 26.4°, resulting in the reduction of the pH to 3.97 and of the acidity to 0.73 grams per 100 cc, calculated as anhydrous citric acid. Ammonium carbonate was added at the rate of one gram per liter, which resulted in a final pH of 4.17 and an acidity of 0.71 grams per 100 cc. Otherwise the technique was the same as described above. The batches were inoculated at 15°C. (60°F.), and fermentation was carried out at room temperature, 15° to 30°C. (60-80°F.). Organoleptic tests showed that the wines from musts to which ammonium carbonate had been added were superior to those from the controls. As in the previous experiment, the curves show a greater velocity of fermentation for the musts containing ammonium carbonate (curve A) than for those not containing this salt (curve B).

The starters containing ammonium carbonate began to evolve carbon dioxide sooner than the plain juice starters and the fermentative action was more vigorous as judged by visual examination. To learn whether the maximum acceleration of fermentation was actually due to the addition of ammonium carbonate to the must and not because of the mere addition of a more vigorous starter, a control starter was added to the carbonate-treated must (0.028 percent nitrogen) and a carbonate-treated starter to a control must (0.0016 percent nitrogen.). The ammonium carbonate added was at the rate of 1 gram per liter. Both starters were inoculated with yeast No. 2338, Saccharomyces ellipsoideus Hansen 1. The sweetened juice samples of 26.4° Brix were 2 liters each; the starter, 125 cubic centimeters each. The samples were fermented at room temperatures, 20°-30°C. (68°-86°F.). The plain must inoculated with the treated starter began fermenting earlier than the treated must inoculated with the plain starter, but in about 17 hours the treated must caught up to the plain must, at which time the carbon dioxide losses were equal. After 17 hours the loss of carbon dioxide of the treated must was greater than that of the plain must, showing a more vigorous fermentation.

The results, based on Saccharomyces ellipsoideus Hansen 1, give, when plotted, curves almost identical with Figure 1, A and B. The data, although not conclusive, since only one experiment was undertaken, agree with those of Lindner[11] and Pringsheim[2] on the optimum concentration of ammonium salts for maximum yeast growth and activity. Maximum acceleration of fermentation was due to the addition of the optimum amount of nitrogenous nutrient and not because of the addition of the more vigorous starter.

Estimation of Yeast Crop: The yeast crop was estimated by the Mueller-Thurgau method[6]. The fermented wines obtained from the two-liter batches of musts were vacuum filtered after addition of 2 percent of a filter aid. The cake was washed with 500 cc of distilled water and then with 500 cc of 180 proof alcohol, followed by drying at 98°C. for 16 hours. An equal volume of unfermented sweetened orange juice was given the same treatment. The difference in weight of the two cakes gave the weight of the yeast crop on a dry basis. The values are approximate and therefore can be used only for comparative purposes (Table 1).

Addition of Converted Ground Distillers' Malt to Orange Must: The malt was finely ground, and distilled water was added in the ratio of 4-1. The malt was converted by heating at 63°C. (145°F.) for 15 minutes and then at 67°C. (152°F.) for 5 minutes. Weighed amounts of the slurry were added to the orange must to bring the malt content to 0.5 and 0.75 percent by weight. Sucrose had been added to the juice to increase the Brix to 23.9°, and the must thus obtained had a pH of 3.62 and a total acidity of 0.68 grams per 100 cc calculated as anhydrous citric acid. Sulphur dioxide was added at the rate of 100 p.p.m. Fermentations were carried out at room temperature, 16°-31°C. (60°-88°F.). This experiment consisted of four parts, which may be conveniently indicated as follows:

(1)	Control, no malt added	Starter plain.
(2)	0.5 percent malt.	Starter plain.
(3)	0.75 percent malt.	Starter plain.
(4)	Plain must.	Starter consisted entirely of malt infusion. Thus, the must actually contained 0.5 percent malt, based on the weight of the must.

Thus, 4 differed from 2 only in the fact that the former contained malt added with the starter. The starter, containing more easily assimilable nitrogenous nutrient, began to ferment earlier and more vigorously and for this reason fermentation of the main batch should begin more rapidly. This was found to be the case. Samples 4, 3 and 2 fermented more rapidly than sample 1, and in the order indicated. For the sake of brevity the complete data covering the eight strains of yeast (32 samples) are omitted. Organoleptic tests showed that the wine from juice to which distillers' malt had been added had a better aroma and taste than wine from juice to which no malt had been added. The total acidities of all of the wines were within the range of 0.68 to 0.84 percent, and the volatile acids ranged from 0.017 to 0.036 percent.

Evaluation of Yeast Strains Used: Fermentative activity of the different yeasts varied. This is very probably due, as pointed out by Lindner[13], to the fact that some strains of yeast are better able to assimilate nutrients than others. Based on rapidity of fermentation and final alcoholic content, it was found that some of the yeast strains employed were not adapted to the fermentation of citrus juices. From the results of these experiments, the values of the strains of yeast used may be listed as follows, arranged in order of decreasing usefulness:

No. 2338	Saccharomyces	ellipsoideus	Hansen I.
No. 236	"	"	Bioletti
No. 4134	"	sake Yabe	
No. 4123	"	ellipsoideus	var. Burgundy
No. 4097	"	"	" Champagne
No. 4098	"	"	" Steinberg
No. 104	"	sp. from oranges (Goresline)	
No. 2372	"	spiritus vini	

The difference in fermentative activity is indicated in Figure 1, which gives results for the most active (curves A and B) and the least active (curves E and F) of the strains used.

SUMMARY AND CONCLUSIONS

The fermentation of sweetened orange juice has been stimulated by the addition of such nutrient materials as ammonium carbonate and distillers' malt. The most favorable range of concentration of ammonium carbonate has been found to be between 0.9 and 1.0 gram per liter; that of distillers' malt to be 0.5 percent when the starter consists of a malt infusion. Because of the inconvenience of preparing distillers' malt, ammonium carbonate would be more suitable.

The aroma and taste of the new wine from must to which ammonium carbonate had been added as a yeast nutrient was better than that of the wine from control batches to which no nutrient salt had been added.

Eight strains of yeast were studied in the fermentation of orange juice. Of those investigated, No. 2338, Saccharomyces ellipsoidous Hansen I, was found to be the most suitable from the standpoint of its vigor and of flavor produced in the finished wine.

Although they have a pleasing bouquet, none of the wines have an odor and taste resembling orange juice.

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Table I - Effect of Ammonium Carbonate (0.9 grams per liter) on Yeast Crop During Fermentation of Orange Must

American Type Culture Collection No.	Name	Yeast Crop Dry Weight in Grams
4098	Saccharomyces ellipsoideus var. Steinberg:	
	With Ammonium Carbonate	9
	Without " "	5
4097	Saccharomyces ellipsoideus var Champagne:	
	With Ammonium Carbonate	8
	Without " "	4
4134	Saccharomyces sake Yabe:	
	With Ammonium Carbonate	12
	Without " "	6
2372	Saccharomyces spirit vini:	
	With Ammonium Carbonate	7
	Without " "	5
4123	Saccharomyces ellipsoideus var Burgundy:	
	With Ammonium Carbonate	12
	Without " "	6

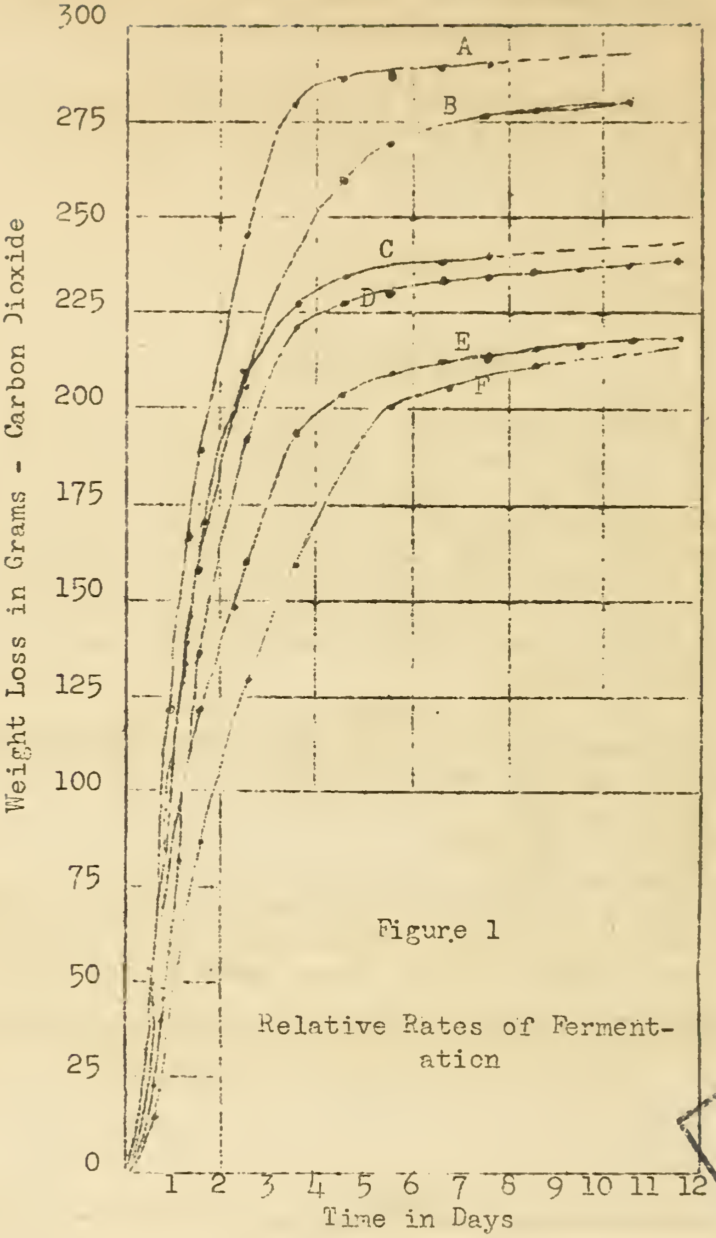

Figure 1

Relative Rates of Fermentation

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A & B
Saccharomyces Ellipsoideus Hansen I
A - Brix 26.4°; pH 4.17; acidity 0.71; $(NH_4)_2\ CO_3$, 1.0 Gram per liter
B - Brix 26.4°; pH 3.97; acidity 0.73; $(NH_4)_2\ CO_3$, none.

C to F
Saccharomyces Spiritus Vini
C - Brix 26.4°; pH 4.17, acidity 0.71; $(NH_4)_2\ CO_3$, 1.0 Gram per liter
D - Brix 26.4°; pH 3.97, acidity 0.73; $(NH_4)_2\ CO_3$, none.
E - Brix 24.4°; pH 4.14, acidity 0.73; $(NH_4)_2\ CO_3$, 0.9 gram per liter.
F - Brix 24.4°; pH 3.97, acidity 0.71; $(NH_4)_2\ CO_3$, none.